CO-SELECTION OF ANTIBIOTIC RESISTANCE IN GRAM-NEGATIVE BACTERIA CAUSED BY POLLUTION LEGACY IN THE CLYDE ESTUARY

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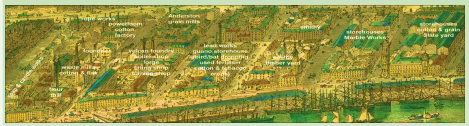
INTRODUCTION



Antimicrobial resistant bacteria can become harboured in sediments of post-industrial estuaries.

Subsequently, their resistance traits could be enriched by pollutants deposited in the sediments. Recent evidence strongly suggests this may pose hazards that not only affects the health care sector, but could also impact tourism and the aquaculture industries.

The River Clyde, UK was chosen for this study due to its extensive industrial history, and three sites were chosen to sample from representing different levels and types of industrial activities—two highly polluted and one relatively "pristine" site.

RIVER CLYDE UK

19 sites of the River Clyde were chosen spanning the estuary in order to obtain a comprehensive overview of the range of pollution in the river.

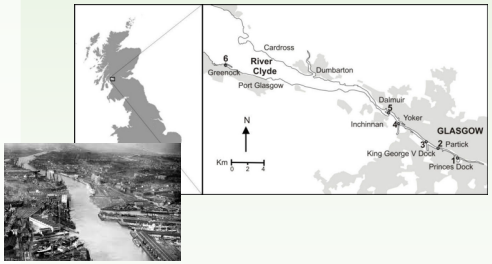


Fig.1 Core depths were taken from all sites using core sampling method shown above and the cores divided into 10cm segments to represent varying time periods in the soil.

OBTAINING ISOLATES

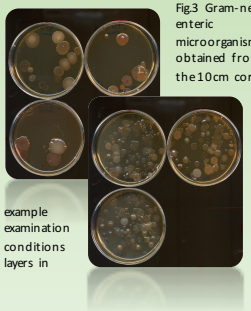


Fig.3 Gram-negative enteric microorganisms were obtained from each of the 10cm core depths.

Fig.2- Core sample example Paleo-limnology is the examination of past environmental conditions through deposition layers in sediment

SUSCEPTIBILITY TESTING

All 19 River Clyde sites were rank summed against Potentially Toxic Elements concentrations found and 3 sites were chosen for susceptibility testing- 2 polluted and 1 pristine. 37 isolates were tested for susceptibilities to an array of PTEs and antibiotics.

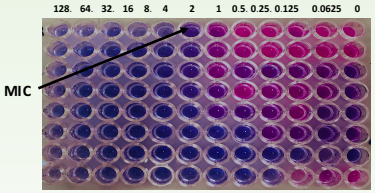


Fig.4 Minimum Inhibitory 96-well assay depicting the resistance of isolated bacteria to selected antibiotic. Values are in two-fold dilution and units are mg/L.

MINIMUM INHIBITORY CONCENTRATIONS

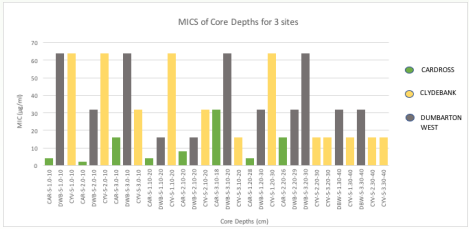


Figure 5- MICs for bacteria isolated from core depths from the 3 sites: Cardross, Dumbarton West and Clydebank.

Higher concentrations of metals in the environment correlated to antibiotic resistance and higher MICs to metals than among bacteria found in less polluted sites. These results also provides some correlation between the absolute levels of the zinc resistant gene ZntA found in the sites via quantitative PCR for the gene.

PCA ANALYSIS

In order to determine the correlation between the potentially toxic elements found within the 3 chosen sites principal component analysis was used. Where the values lie between the 3 components highlights the variation between source of pollutant and how the MICs and MBCs for bacteria isolate correlate.

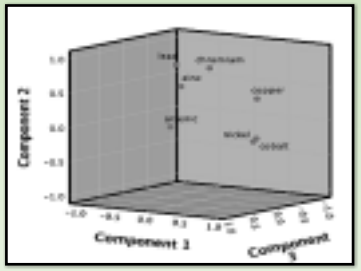


Fig. 6- PCA analysis of the PTE values in Cardross, Dumbarton West and Clydebank

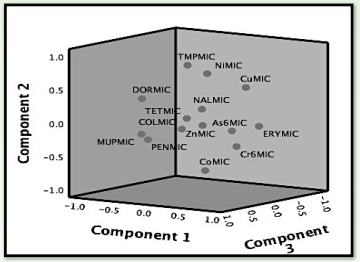


Fig. 7- PCA analysis of the MIC values in Cardross, Dumbarton West and Clydebank

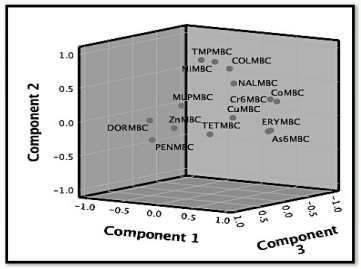


Fig. 8- PCA analysis of the MBC values in Cardross, Dumbarton West and Clydebank, MIC and MBC values

BIIVARIATE ANALYSIS

Toxicant	Bio-Metric	Geochemical Correlations
Copper	MIC	pH (r=.444*), Co(-.630 **), Cu(r=.481*), Ni(.612*)
	IC50	pH (r=.444*), Co(-.630 **), Cu(r=.521*), Ni(.612*)
Nickel	MIC	Co (r=.404*), Cu(-.429 **), Ni(-.359*)
Cobalt	MIC	Co (r=.459*), Cu(-.403*), Ni(-.552**)
	IC50	Co (r=-.332*)
Chromium (6)	MIC	cr6 (r=-.339*)
Penicillin	MIC	As (r=-.330*)
	IC50	As (r=-.388*), Zn (r=-.387*)
Trimethoprim	MIC	Zn (r=.471**)
	IC50	As (r=-.325*), Cu(r=.370*), Zn (r=-.680**)
	MBC	Zn (r=.505**)
Tetracycline	MIC	pH (r=-.386*), As (r=-.377*), Cu (r=-.328*)
	IC50	pH (r=.487**), As(r=-.464**), Co (r=-.431**), Cu (r=-.484*), Ni (r=-.331*)
	MBC	pH (r=-.367*)
Colistin	MBC	Zn (r=-.401**)
Doripenem	MBC	As (r=-.346*), Ni(r=-.383*)

r= Pearson correlation. *sig. <0.050 **sig. <0.005

Fig. 9 Significant bivariate correlations between MIC, MBC and IC50 of toxicants in terms of geochemical conditions. All variables were log2 transformed prior to analysis for both PCA and Bivariate analysis.

HIGH-THROUGHPUT GENE ARRAY QPCR

Applied Biosciences gene-chip analysis was carried out on the 3 sites chosen for the susceptibility assays.

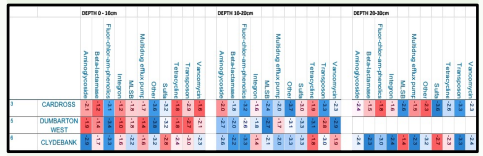


Fig.10 Data shows the presence of resistance gene profiles in core depths obtained from each site.

All values represent genes/16S-rRNA (total bacteria) that have been log-transformed (eg., -1 =10%, -2 = 1%, -3 =0.1% population with the gene).

CONCLUSIONS

- From the results obtained it is clear that gram-negative bacteria isolated from an area with an extensive industrial pollution history show higher minimum inhibitory concentrations and minimum bactericidal concentrations to a range of both PTEs and antibiotics.
- In general, genes for resistance mechanisms were shown to be highest within 0-10cm of soils however when examining data from deeper cores, isolated bacteria still harbour resistance traits to both PTEs and antibiotics.
- Through a combination of susceptibility assay data, qPCR and high throughput gene array qPCR technology and, it is clear co-selection of PTEs and antibiotic resistance does occur, and this impacts bacteria that are potential human pathogens.